Promising potential of bio-sourced amphiphilic xanthan as an emulsifier in O/W dispersions

Mira Abou Dib, Marie Lemarchand, Ecaterina Gore, Michel Grisel

Promising potential of bio-sourced amphiphilic xanthan as an emulsifier in O/W dispersions

Mira Abou Dib*, Marie Lemarchand, Ecaterina Gore, Michel Grisel*

Normandie Univ, UNILEHAVRE, FR 3038 CNRS, URCOM, 25 rue Philippe Lebon, BP 1123, 76063 Le Havre cedex, France

**corresponding author: michel.grisel@univ-lehavre.fr and mira.aboudib.27@gmail.com*

Abstract

The emulsification properties of hydrophobically modified xanthan (HMX) obtained through green esterification using alkenyl succinic anhydrides (ASA) as grafting agents were investigated by preparing oil-in-water emulsions without adding any surfactant, in order to better understand the stability phenomenon at the oil-water interface. This study demonstrated that new-brand bio-sourced HMX owns emulsifying potential compared to pristine xanthane, known for its poor interfacial properties as a non-absorbing polymer. A complete approach for studying the stability of the dispersions was carried out at different levels: a) macroscopic level: visual identification of stability, b) microscopic level: coupled optical microscopy and droplet size distribution and c) deep microstructure stability monitoring over time by Static Multiple Light Scattering. First, a series of HMX differing in alkenyl chain length and grafting density added at 0.2% w/w in emulsions were compared. Increased grafting density or hydrophobic chain length provided a highly stable and homogeneous emulsion over 30 days. In the case of less stable emulsions, two solutions are proposed: 1) increasing HMX concentration thus improving emulsifying properties, 2) adding native xanthan as a thickening agent, which slows down the creaming destabilization mechanism. This original article shows that the new-brand bio-sourced amphiphilic xanthan obtained under green modification synthesis behave promising and effective emulsifying capacities to stabilize oil-in-water emulsions, which offers promising potential for industrial applications.

Keywords

Amphiphilic xanthan, green modification, oil-in-water dispersion, stabilization, emulsification properties

1. Introduction

Emulsions are widely used and developed in multiple industrial fields such as foods [1], cosmetics [2], pharmaceuticals [3] and agrochemicals [4]. They are thermodynamically unstable mixtures made of two or more immiscible liquids [5]; oil-in-water (O/W) emulsions consist of oil droplets dispersed in a continuous aqueous phase. Since the contact between oil and water is energetically unfavorable, O/W emulsions tend to undergo destabilization processes over time such as creaming, flocculation, coalescence and Ostwald ripening; thus leading to the oiling off and the emulsion breaks up into phase separation [6,7]. However, it is possible to kinetically stabilize emulsions by adding additional components that can be: a) thickening agents such as polysaccharides that slow down droplets motion and breaking phenomenon thanks to their ability to increase viscosity of aqueous phase [8] and b) emulsifiers such as surfactants [9], solid particles [10] or polymeric surfactants [11–13]. In fact, the

emulsifier can adsorb at the oil-water interface providing a protective barrier that prevents or slows coalescence, ensuring long-term stability. Among polymeric surfactants, despite their high interest due to natural origin, there is only a limited number of polysaccharides naturally owning surface activity and emulsifying properties such as Arabic gum [14], talha gum [15], mesquite gum [16] and certain galactomannans such as guar, locust bean [17,18], and fenugreek gum [19,20] which surface activity was attributed to the hydrophobic protein component that anchor strongly the oil-water interface, while the hydrophilic hydrocolloid chains form protective layer providing steric stabilization [11]. Many other polysaccharides lack surface activity and do not exhibit adsorbent character so they cannot bring any interfaces stabilization. To deal with this poor interfacial properties of such hydrocolloid, multiple studies were developed on hydrophobically modification of polysaccharides based on the pioneering work of Landoll [21] to endow them with interfacial properties. Among other polysaccharides, xanthan is well known for its outstanding viscosifying capacities that can lead to dispersions stabilization due to thickening effect of the continuous phase [22]. Nevertheless, its poor interfacial properties prevent it to long-term stabilize dispersions without adding conventional surfactants; however these latter compounds own negative toxicological and environmental impacts [23,24].

Recent literature studies have shown that ASA-modified polysaccharides have different potential applications mainly encapsulation, emulsification and stabilization [25–29]. In addition, one of them was approved as additive in food and medicine by the Food and Drug administration, it is the octenyl grafted starch (OSA-starch) [30].

In a previous work, a variety of amphiphilic xanthan derivatives were prepared via green esterification reaction between xanthan and a series of alkenyl succinic anhydride (ASA) as grafting agents [31]. The study allowed revealing optimized conditions to obtain high grafted xanthan with three different alkenyl chain length, namely octenyl, dodecenyl and hexadecenyl; it also highlighted the surface activity enhancement of the xanthan when grafted with long alkenyl chain or high rate of hydrophobic moieties.

The present work details the potential for amphiphilic xanthan derivatives to emulsify dispersions without adding any low molecular weight surfactant. The study first focuses on the effect of the grafting density and the grafted chain length on the potentiality to stabilize O/W emulsions. Then, the impact of HMX concentration as well as its synergy with native xanthan is described. Properties of all emulsions was evaluated and compared over time in order to understand the phenomenon governing the emulsions stability.

2. Materials and methods

2.1. Materials

Xanthan gum sample was kindly given by Cargill (France) and characterized in terms of moisture content (11.3% w/w), protein content (0.95% w/w) and substitution degrees of acetate and pyruvate per monomeric unit, 0.56 and 0.59 respectively [31]. Hydrochloric acid (HCl) was purchased from Fisher Scientific (USA), sodium chloride (NaCl) from Carlo Erba and sodium azide (NaN_3) used as preservative against bacteria growth was from Sigma Aldrich (USA). Ultra-pure water (16.8 MΩ.cm resistivity) was used as solvent for all preparations. For emulsion preparation, isononyl isononanoate (ININ) was used as oil phase with a density of 0.856 g/cm^3, it was kindly offered by Stéarinerie Dubois (France). All chemical reagents were analytical grades.

2.2. Synthesis of hydrophobically modified xanthan

Hydrophobically modified xanthan HMX samples were obtained via a succinylation reaction of xanthan using 2-Octen-1-ylsuccinic anhydride (OSA), (2-Dodecen-1-yl) succinic anhydride (DDSA) or Hexadecenyl succinic anhydride (HDSA) based on a procedure according to [31].

As fully described in a previous study, reaction optimization was performed for each HMX derivatives (C8, C12 and C16) by varying either the reaction pH range, the reaction time and the grafting reagents molar excess thus allowing accurate control of degree of succinylation (DS) up to 16 %, as determined by 1H NMR analysis [31]. All HMX used in this study remained fully water soluble thus allowing pouring back in water. Resulting HMX were noted as XC8, XC12 and XC16 for octenyl-grafted, dodecenyl-grafted and hexadecenyl-grafted xanthan, respectively, followed by their DS value. As an example, a xanthan modified by OSA with a DS of 4.1 % will be noted as XC8(4.1). Pristine xanthan (PX) refers to samples subjected to the same treatment apart from adding OSA, DDSA or HDSA. Native xanthan (NX) represent the xanthan commercial batch.

2.3. Protocol for O/W emulsion preparation

Emulsions preparation was adapted from the optimized protocol previously developed in our laboratory for preparing small emulsions quantities with efficient and reliable procedure [32]. Thereby, it was possible to prepare in a controlled and perfectly reproducible manner 20 g of O/W emulsion containing of 20 % (w/w) of oil phase (w/w) dispersed in 80 % (w/w) of aqueous phase. The mass composition of the emulsions is detailed in Table 1.

For the aqueous phase, xanthan (native, pristine or modified) was first dissolved at concentration ranging from 0.25 to 0.625 % w/w in ultrapure water under magnetic stirring at room temperature overnight. Afterwards, sodium azide NaN_3 used as preservative is added at 300 ppm in the solution to avoid any bacterial contamination. Then, the pH was adjusted to 4.3 by adding 0.1 M HCl and the ionic strength to 1 mS/cm with NaCl(s).

Then, the fatty phase solely consisting of ININ oil was gently poured into the aqueous phase. The two immiscible phases were heated in a water bath at 30°C in order to fully control the temperature of the dispersion process. Once 30°C was reached, the mixture was homogenized for 3 minutes at 20 000 RPM using a T25 digital Ultra-Turrax (IKA, Germany) equipped with the dispersion head S25N-10G. Resulting dispersion was then immediately degassed under vacuum in order to eliminate the air incorporated during the homogenization process prior to any analysis.

Each dispersion was prepared in duplicate in order to verify the protocol control and reliability, and also to get sufficient dispersion quantity to allow performing a series of physicochemical characterizations and complementary studies.

Table 1 : Composition of the formulation

Product	Content % (w/w)	Phase
Isononyl isononanoate ININ	20	Oil
Xanthan (NX, PX or HMX)	0.2 to 0.5	Aqueous
Preservative NaN_3	0.025	
Ultrapure water	QS	

2.4. Methods for the characterization of dispersions

On the one hand, a study based on visual macroscopic identification of stability/separation followed by the microstructure observation and analysis of both droplet size and distribution in every identified zone was assessed over the whole period of ageing. From a general point of view, emulsions carried out with xanthan derivatives generally showed macroscopic phase separation into two phases: an aqueous lower phase and an emulsified upper phase. In some cases, mixtures showed a third upper phase consisting of non-emulsified supernatant oil.

On the other hand, an optical automated study of the entire volume of dispersion was considered by analyzing the percentage of transmitted and backscattered light through dispersion as obtained by Static Multiple Light Scattering measurement. Such an experimental approach allowed monitoring and then understanding the different destabilization phenomenon involved in emulsions ageing, namely creaming, flocculation and coalescence, thus being complementary from visual and microscopic observations of the dispersions.

Characterizations was made directly after emulsification (D0) then over time (up to 30 days). The designation D(i) will be applied referring to (i) the day of the characterizations. Only selected days are showed in this article. Samples were stored at 20 °C for the entire duration of the study.

2.4.1. Macroscopic observations

The macroscopic aspect of the emulsions was followed by digital photographs just after formulation and over time. Thus, it is possible to evaluate the emulsifying capacity of HMX and to detect the macroscopic destabilization phenomena during aging.

2.4.2. Optical microscopy

Emulsion microstructure was observed, at room temperature with an optical microscope at a magnification ranging from x100 up to x400 (ECLIPSE Ni-U NIKON, Japan) equipped with a camera DS-Fi3 (5.9 megapixels CMOS image sensor). The NIS-Elements L imaging software was used to analyze the photomicrograph.

2.4.3. Droplets size distribution

Emulsion droplets size was measured by static light scattering using a laser diffraction particle size analyzer SALD–7500 nano (Shimadzu, Japan) equipped with a violet semi-conductor laser (405 nm) and a reverse Fourier optical system. In order to study destabilization phenomena, a volume of approximately 5 µL taken from the center of each of zone described above (upper and lower) was diluted in ultrapure water prior to measurement in order to achieve absorption parameter within the interval between 0.16 and 0.20. Sample was homogenized in the measuring batch cell (7 mL) using mechanical stirring. Three repetitions were performed for each measurement using Fraunhofer's theory. Data were analyzed using software Wing SALD II. Characterizations of the upper part of the sample leads to a study of emulsion droplets size evolution during aging. However, characterizations of the lower part were only used to confirm that less droplets remain in this separated phase.

2.4.4. Stability of the dispersion by Static Multiple Light Scattering technique (SMLS)

The stability of O/W emulsions is also characterized by the backscattering (BS) and transmission (T) of a pulsed near infrared light (880 nm) by Turbiscan™ Tower (Formulaction, France) which is a technique

based on multiple light scattering. The basic principle for such an apparatus is as follow: standardized glass tube containing the sample is locally illuminated by infrared light moving on the entire sample height from the bottom to the top of the volume; two synchronous optical sensors respectively receive: on the one hand the light transmitted through the dispersion at 180° angle from the incident light (T detector), and on the other hand light scattered backward by the dispersion at 45° from the incident radiation (BS detector) (Figure 1a). Both the collected T and BS data allow the detection of the migration phenomena of droplets and their size changings in dispersion revealing the presence of the different types of destabilization processes currently corresponding to droplets flocculation, coalescence, creaming and/or sedimentation which lead to a phase separation [33]. In general, transmission is used to analyze clear to turbid dispersions and backscattering to analyze opaque and concentrated dispersions.

The BS profile (vertical view) in Figure 1b shows an increase of BS in the low concentrated zone of the sample while a decreasing of BS indicating clarification corresponds to the top diluted zone. Such behaviors are proper to the sedimentation of droplets. The T profile might show opposite variation in each zone of the same sample (profile not shown to ensure clarity).

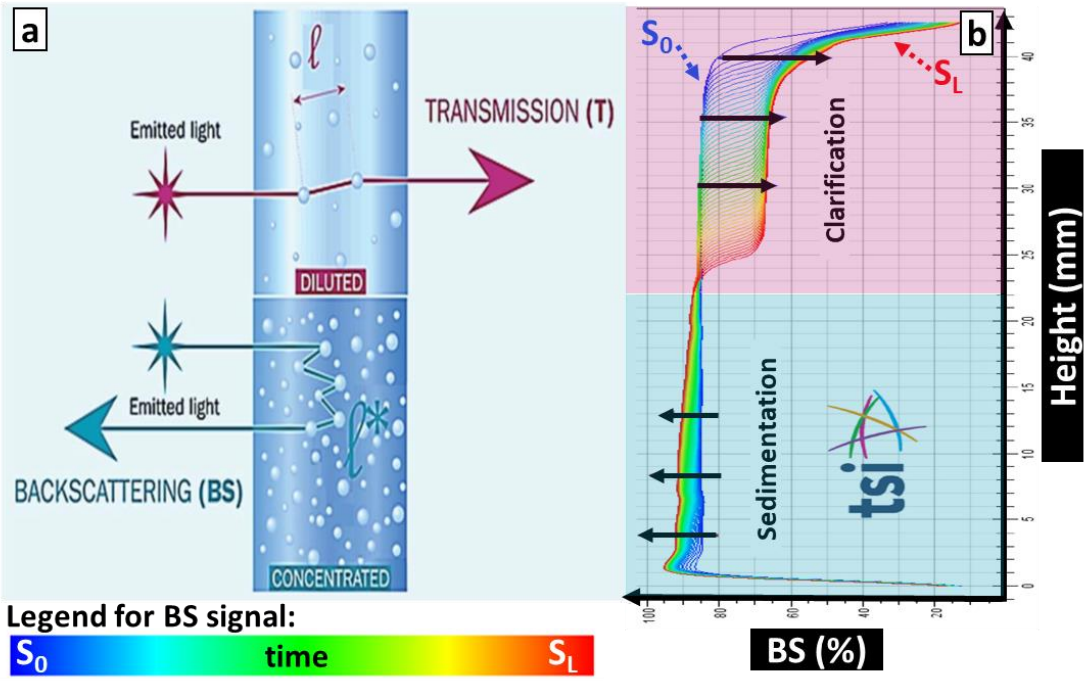

Figure 1: a) Work principle of SMLS-TURBISCAN™ Tower: glass tube containing dispersion with b) backscattering profile (vertical view) showing sedimentation phenomena (right), adapted from [12,34]

Both the transmitted (T) and backscattered (BS) flux, are related to the photon mean path length λ and mean free path λ^*, respectively, as follows [34,35]:

$$T = T_0 e^{-\frac{2r_i}{\lambda}} \quad (1)$$

$$\lambda(d, \emptyset) = \frac{2d}{3\emptyset Q_s} \quad (2)$$

$$BS = \frac{1}{\sqrt{\lambda^*}} \quad (3)$$

$$\lambda^*(d, \emptyset) = \frac{\lambda}{1-g} = \frac{2d}{3\emptyset(1-g)Q_s} \quad (4)$$

Where:

- r_i is the internal radius of the measurement cell;
- T_0 is the transmittance of the continuous phase;
- d and $\emptyset$ are the mean diameter and the volume fraction of droplets, respectively;
- g is the asymmetry factor and Q_s corresponds to the scattering efficiency factor (both are optical parameters given by the Mie theory).

Immediately after dispersion preparation, standardized measuring cylindrical glass cells was filled with 15 g samples to be analyzed at 20 °C. The entire height of the studied samples was scanned over time (D0 to D30). Results were registered by TowerSoft (version 2.0.0.9). Both the transmitted and backscattered profiles along the height of the cell were collected as raw data.

Emulsion stability can be estimated using the Turbiscan Stability Index (TSI) as proposed by the software. TSI compares each scan (i) to the previous one $(i-1)$ at a selected height (h), thus dividing the result by the total height(H) as indicated below [34,36]:

$$TSI = \sum_i \frac{\sum_h |Scan_i(h) - Scan_{i-1}(h)|}{H} \quad (4)$$

Thus, TSI index gives access to the kinetic of the destabilization; it was conceived to simplify the comparison between samples. Therefore, the lower the TSI value at a particular time, the more stable is the dispersion.

Among other advantages when compared to other optical methods currently used (including some used in the present paper), SMLS technique is a non-destructive method (no sampling or dilution), it detects destabilization phenomenon at a very early stage allowing to shorten aging tests and it provides together the identification of particle migration (creaming, sedimentation) and variation size variation or aggregation (flocculation, coalescence) [35].

3. Results and discussion

3.1. Evaluation of the emulsifying capacity

First, preliminary experiments using 0.2% of pristine xanthan in the O/W mixture demonstrated as expected that no emulsion stabilization was obtained, thus leading to a spontaneous phase separation. In fact, no droplets were detected by microscopic analysis immediately after the preparation process. This result is similar to that also obtained with native xanthan confirming that xanthan behaves as a non-absorbing polysaccharide due to poor interfacial properties [37,38]. However, when replacing PX by XC8(4.1), a low grafted xanthan modified with a short alkenyl chain, one can notice that emulsion was formed on the entire height of the mixture, showing large droplets as evidenced by optical microscopy image as illustrated by Figure 2. Indeed, at D0 the emulsion looks white and homogeneous from a macroscopic point of view with almost all the fatty phase dispersed in the aqueous phase. However, a thin supernatant layer of oil was rapidly observed indicating insufficient emulsifying capacity of this HMX sample at the studied concentration (0.2%). The study of the system after 24 h showed obvious phase separation with mainly two distinct phases: an upper white one for roughly ⅓

of the mixture height and a lower turbid one with ⅔ of the height (images not shown). After one month, one can see that the two phases remained present with almost the same phases proportions as observed after 1 day, thus indicating good creaming stability during storage (Figure 2). The microstructural monitoring of both phases from D0 to D30 showed that no droplets remains in the lower phase, corresponding to the aqueous one; on the contrary within the upper emulsified phase, oil droplets of heterogeneous size still remained dispersed.

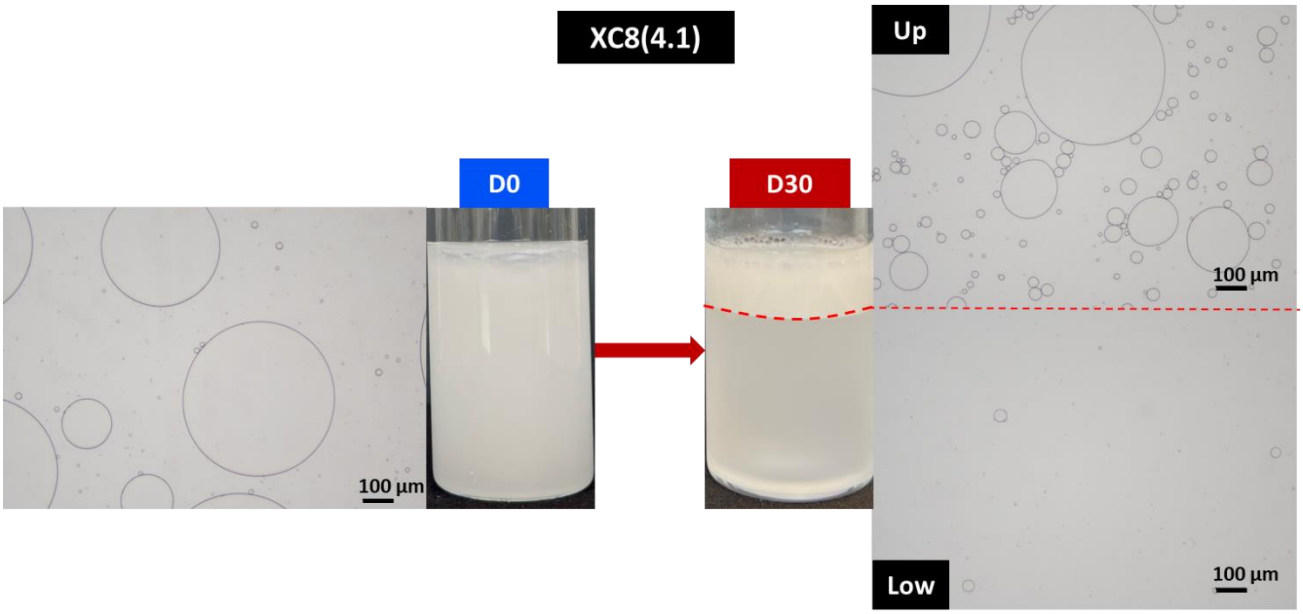

Figure 2 : Photographs and optical microscopy image of emulsion at 0.2% w/w XC8(4.1) at D0 and D30

One can also note that when compared to the microscopic image at D0, the one at D30 showed a higher number of smaller droplets which was confirmed by a decrease of the mean diameter determined by static light scattering measurements performed for the emulsified phase over time (Figure 3a). This may simply result from the migration of droplets from the bottom to the top corresponding to the presence of creaming phenomenon.

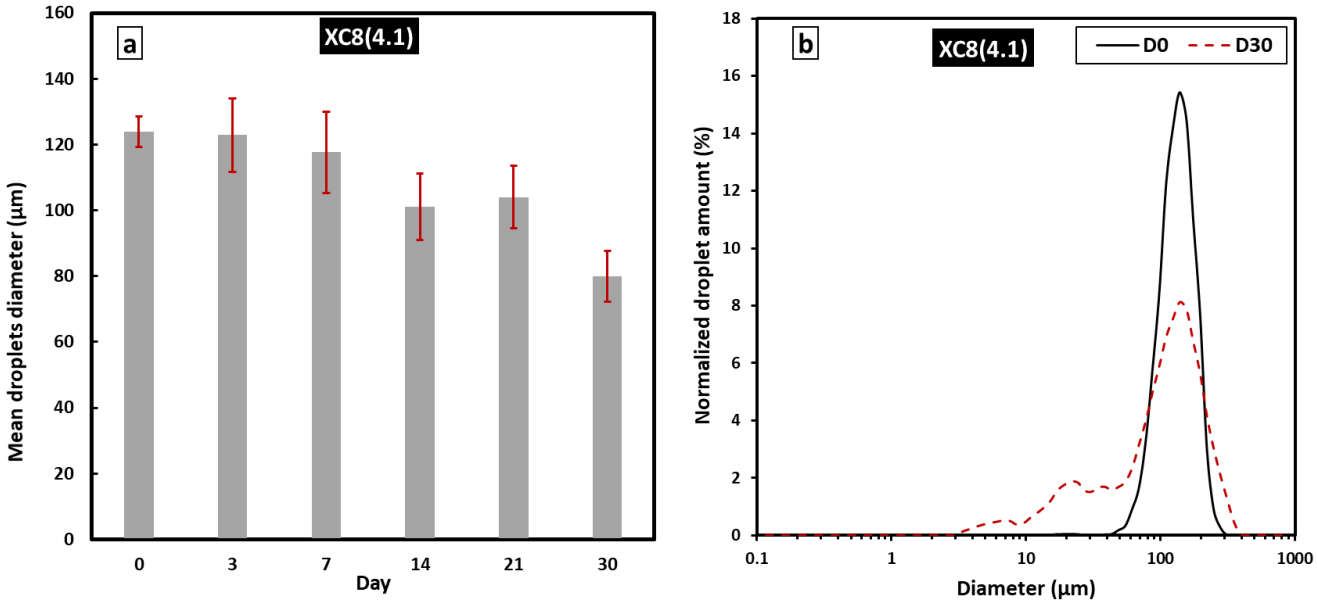

Figure 3 : Evolution of: a) mean droplets diameter of the emulsified phase at different time after preparation, b) droplets size distribution at D0 and D30; for emulsion at 0.2% w/w XC8(4.1)

In order to better understand the microstructure destabilization, it was necessary to analyze the entire graphs of droplets size distribution of the emulsified phase. As presented in Figure 3b, the size distribution of the emulsified phase, initially monomodal and monodisperse (D0), becomes multimodal after aging with a polydisperse distribution (D30). This can be explained by two simultaneous mechanisms: 1) the flocculation of the droplets as a consequence of spontaneous instability (i.e. insufficient number of HMX absorbing chains present at the oil-water interface and lack of electrostatic repulsions between the droplets) and 2) the creaming from the bottom to the top with gravitational migration of almost all droplets including the smaller ones, into a restricted volume (⅓ of the height) corresponding to the oil concentrated phase. Nevertheless, the resulting heterogeneous distribution of stabilized droplet as evidenced through granulometric measurements clearly confirms the microscopic visualization.

All these observations allow drawing hypothesis on the destabilization phenomenon that could have occurred in the system. However, the need for more precise method remains essential to check the interpretations. To this purpose, Turbiscan™ tower was used to allow a monitoring over short periods (until 1 scan every 2 minutes) of the entire XC8(4.1) dispersion height; resulting backscattering and transmission signals are presented in Figure 4. Analyzing both BS and T profiles at D0 (first blue curve) showed constant value of BS and no transmission percentage over 30.5 mm height thus indicating mainly homogeneous distribution of the emulsion. Then for the upper 2.5 mm, a decrease of BS and an increase of T were observed, revealing a clarification due to the supernatant oil phase; these results allow confirming the interpretation described above based on visual observation. Afterwards the monitoring over 1 month allows the identification of two principle behaviors: first, a decrease of BS (up to 2%) together with an increase of T below 24.5 mm height indicating a clarification of the lower aqueous phase; simultaneously, an increase of BS with no transmission indicating an opacification of the upper emulsified phase (24.5-30.5 mm). Both simultaneous phenomena clearly allow demonstrating the progressive occurrence of creaming destabilization mechanism. Concerning, the upper thin zone [30.5-33 mm], so-called supernatant oil phase, it would be difficult to delimit it precisely because it is located between the emulsified active phase and the meniscus.

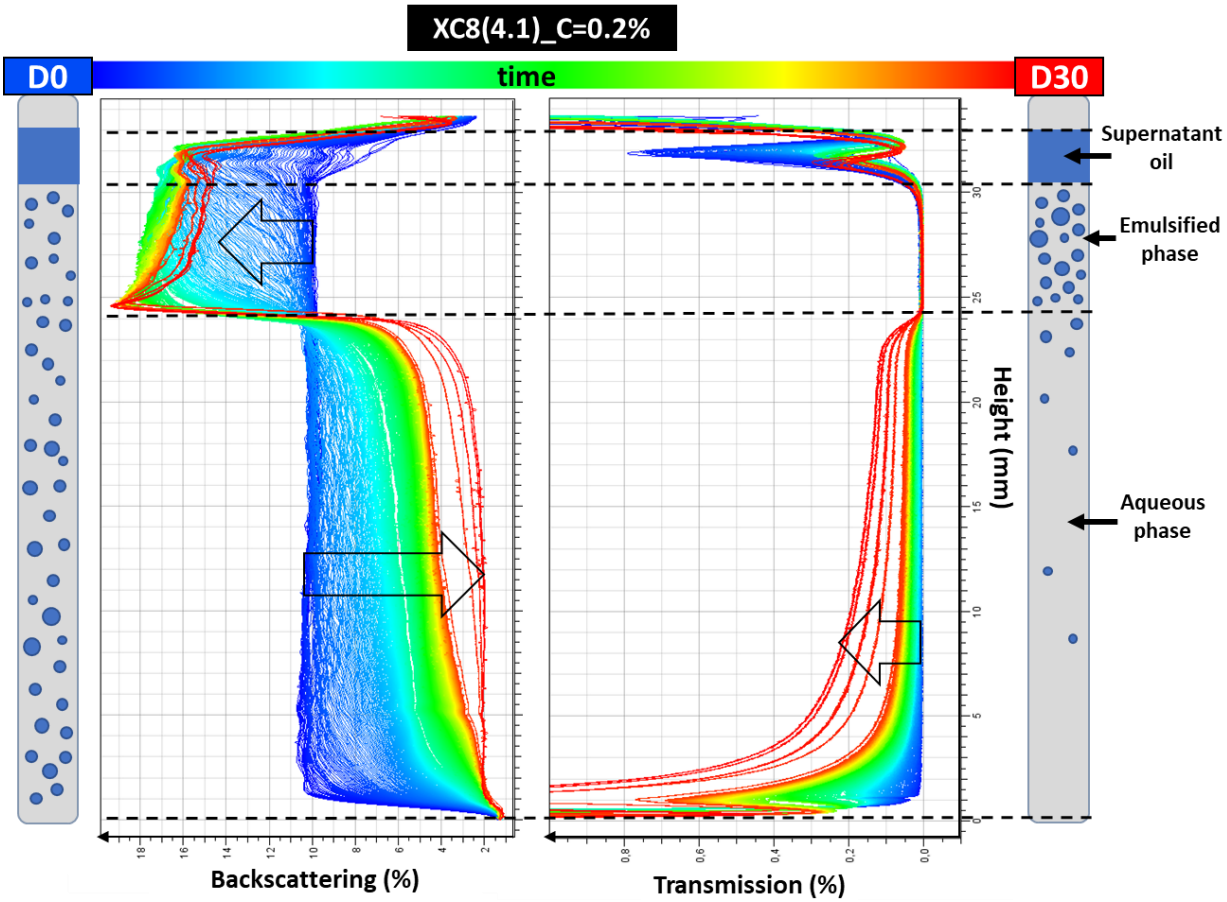

Figure 4 : Raw backscattering and transmission data obtained by following the evolution with time of the destabilization of the emulsion formed with XC8(4.1) at 0.2% w/w. The sample was scanned over 30 days with different scan periods

In this first section, the emulsifying capacity of a single HMX sample was evidenced and the destabilization phenomenon governing the evolution of the dispersion over time were analyzed and interpreted. In fact, the interface stabilizing character of this specific HMX owning low DS (4.1%) of short alkyl residues (octyl) remains insufficient in the tested conditions (polymer concentration of 0.2% w/w) to get full stabilization of the dispersion. So, next sections will aim studying the impact of each parameter, namely DS, alkyl chain length and polymer concentration, on improving the emulsifying potential.

3.2. Impact of DS and alkenyl chain length on emulsifying capacity

In this part, all emulsions were prepared using HMX at 0.2% (w/w) with varying both DS and alkenyl residue. In these conditions all emulsions appeared white and homogeneous upon preparation procedure. Both macroscopic and microstructure aspects were determined and monitored over 30 days in order to investigate the ability for HMX to get long-term stability emulsion in thus allowing comparing all systems together.

Only photographs corresponding to D30 for all mixtures were presented and compared in order to visualize the corresponding macroscopic stability over 1 month (Figure 5). One can notice for XC8 that using HMX owning a higher DS (6.2% rather than 4.1%) do not significantly improve its emulsifying capacity. Nevertheless, using XC12 instead of XC8 with keeping constant DS (6.2-6.3%) clearly allows reaching much higher stability as the entire volume of the dispersion appears emulsified from a macroscopic point of view. Same observation was obtained with XC12(16.9) (results are not shown to

keep clarity). However, it is striking that, below a certain DS, XC12 does not bring sufficient emulsifying potential to stabilize the entire system in the studied conditions (C=0.2%), as illustrated for XC12(2.2) in Figure 5. Finally, utilizing HMX grafted with long alkenyl chains (C16), although the grafting density is low (DS = 2.2%), allows obtaining a macroscopically stable emulsion.

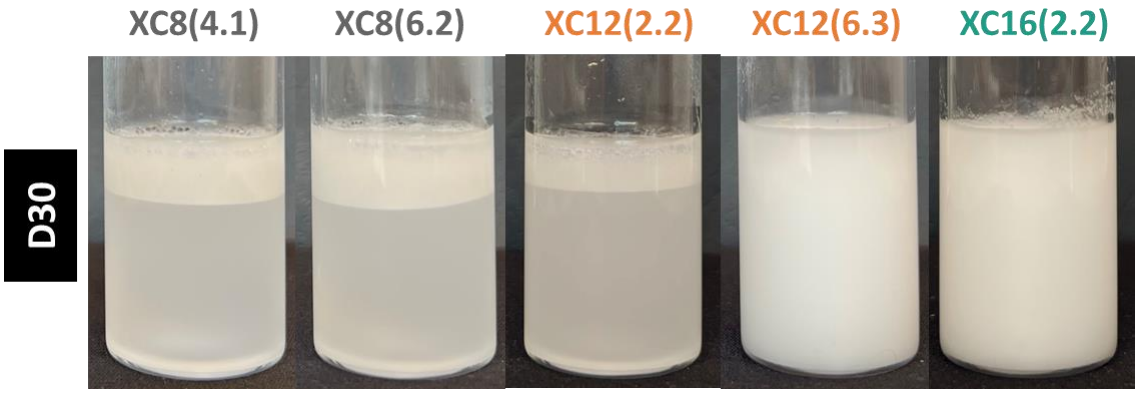

Figure 5 : Photographs of emulsions at 0.2% w/w of HMX with different alkenyl chain length and grafting densities

It is obvious that macroscopic observations cannot be sufficient for understanding and interpreting the mechanisms responsible for emulsions stability with varying HMX structure. Thus, the same methods for investigation as the one used for section 1 of this paper were envisaged in order to allow accurate interpretations on the basis of microstructure considerations. Indeed, Figure 6 gives the optical micrographs of emulsified phase at D30 superimposed with the droplet size distributions graphs for the series of emulsions at different aging stages, D0 and D30 respectively.

Comparing microphotographs shows that all samples present droplets in the emulsified phase 30 days after the dispersion preparation, thus confirm that HMX own emulsifying properties whatever the alkenyl chain length and DS. Then, one can notice that the higher the DS (> 6%) or the longer the alkenyl chain length (C16), the higher the density of droplet and the smaller their size (Figure 6 b,d,e). This clearly indicates an improvement of the stabilizing character due to both DS and grafted chain length. Considering the droplet size distribution, particular behaviors are obtained for both XC12(6.3) and XC16(2.2) owning the distribution at D0 and D30 perfectly superimposed with droplets mean and median diameters remaining constant around 10 to 20 µm as indicated in Table 2. Based on these results, one can conclude that HMX specimen allow preparing stable emulsions within the period of time tested as no destabilization was observed within 1 month. Nevertheless, one can notice that slight difference actually exists between microphotographs as some of the droplets of XC12(6.3) emulsion (Figure 6d) have a less spherical shape when compared to those for XC16(2.2) emulsion (Figure 6e). Such a qualitative observation can be explained by the occurrence of flocculation phenomenon. This hypothesis may could be checked on the basis of further analysis such as backscattering data.

For the other emulsions analysis, the evolution of the droplet size distribution curves (Figure 6a,b,c) showing a decrease of both median and mean diameter of the droplets (Table 2) reveal that destabilization mechanisms occurred between D0 and D30 mainly by creaming together with a limited phenomenon of flocculation or even the absence of flocculation, this according to the grafting density and the length of the alkyl residue. These latter results and interpretations are in good accordance with the ones discussed in the first section for XC8(4.1).

Similar effect of alkenyl chain length was discussed by Kokubun et al. [39], where emulsion droplet size was smaller for DDSA-inulin than OSA-inulin owning equivalent DS. This indicated that longer hydrophobic moieties make it easier polymeric species adsorption onto oil droplets during the homogenization process.

If considering the impact of DS, emulsion stability improvement and smaller droplet size were attributed to higher number of hydrophobic moieties as demonstrated by Kokubun et al. [39] and Wang et al. [29] on inulin-OSA/DDSA and Arabic gum-DDSA respectively.

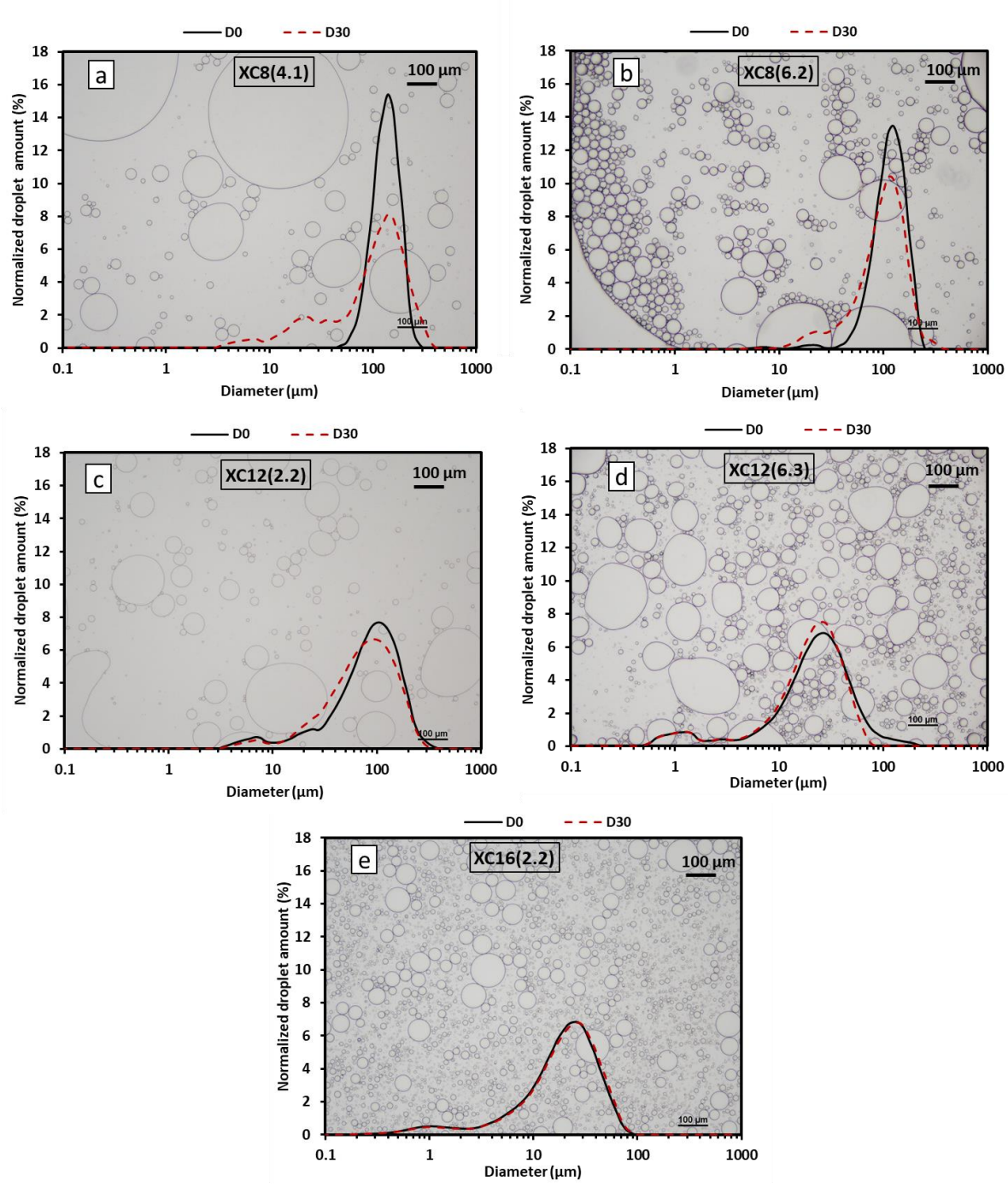

Figure 6 : Optical microscopy image at D30 and droplet size distribution at D0 and D30 of the emulsified phase of dispersion at 0.2% w/w of different HMX

Table 2 : Median and mean diameter of droplets in the emulsified phase of dispersion at 0.2% w/w of different HMX between D0 and D30

		XC8(4.1)	XC8(6.2)	XC12(2.2)	XC12(6.3)	XC16(2.2)
Median diameter (µm)	**D0**	126 ± 5	110 ± 2	83 ± 5	22 ± 1	19 ± 1
	D30	105 ± 8	96 ± 16	72 ± 3	20 ± 1	20 ± 1
Mean diameter (µm)	**D0**	124 ± 5	105 ± 2	70 ± 6	18 ± 1	15 ± 1
	D30	80 ± 8	87 ± 17	63 ± 2	16 ± 1	16 ± 1

Following the same procedure as the one described in section 1, Turbiscan™ characterization of the different samples was conducted over 1 month. Since the transmission percentage is lower than 0.2%, the analysis of SMLS was focused on BS signal. Results allowed evidencing two distinct behaviors. The first one observed for both XC8(6.3) and XC12(2.2) evidenced creaming quite similarly as the profile obtained for XC8(4.1) (profile not shown - see Figure 4 for comparison). Only slight difference could be evidenced for the height of the emulsified phase, but the variation clearly remains non-significant. The second type of profile shows constant BS value which perfectly superimposes on the entire height of the sample over the whole period of sample monitoring (Figure 7a,b) corresponding to XC12(6.3) and XC16(2.2), respectively. Same results were observed for XC12(16.9) (data not shown). Thus, no destabilization occurs and a relatively high and constant opacity (BS = 43-45%) is observed for these last samples thus illustrating without any doubt the occurrence of highly stable emulsions.

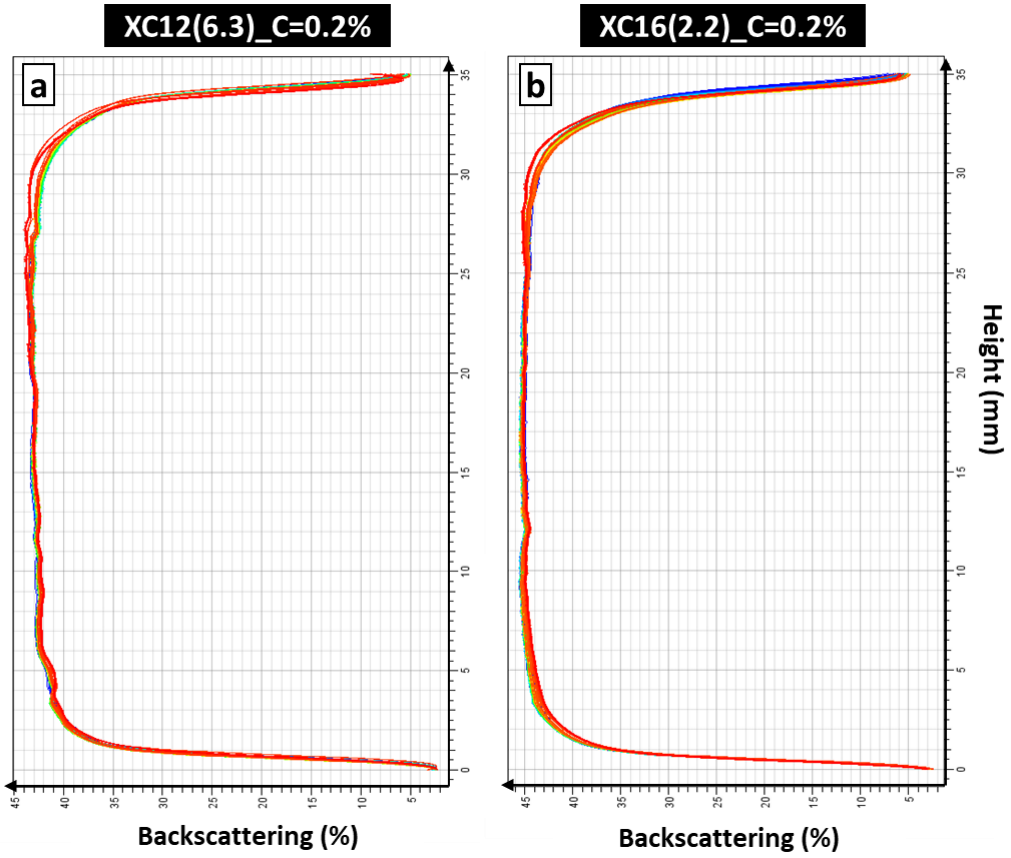

Figure 7 : Raw backscattering data obtained by following the evolution with time of the destabilization of the emulsion formed with: a) XC12(6.3) or b) XC16(2.2) at 0.2% w/w. Samples were scanned over 30 days with different scan periods

In addition to the results exposed and discussed above, further parameter could be analyzed from Turbiscan™ data, namely the destabilization kinetic of the system as evidenced through the evolution of TSI index as a function of time. According to Figure 8, a relatively fast destabilization occurs even from the first hours after preparation for emulsions defined as unstable after one month. Nevertheless, TSI evolution appears slower for HMX owning high DS or long alkenyl grafted moieties as in these latter cases a low index value (TSI ≤ 2) is observed over 1 month when compared to HMX owning low DS and short alkenyl residues.

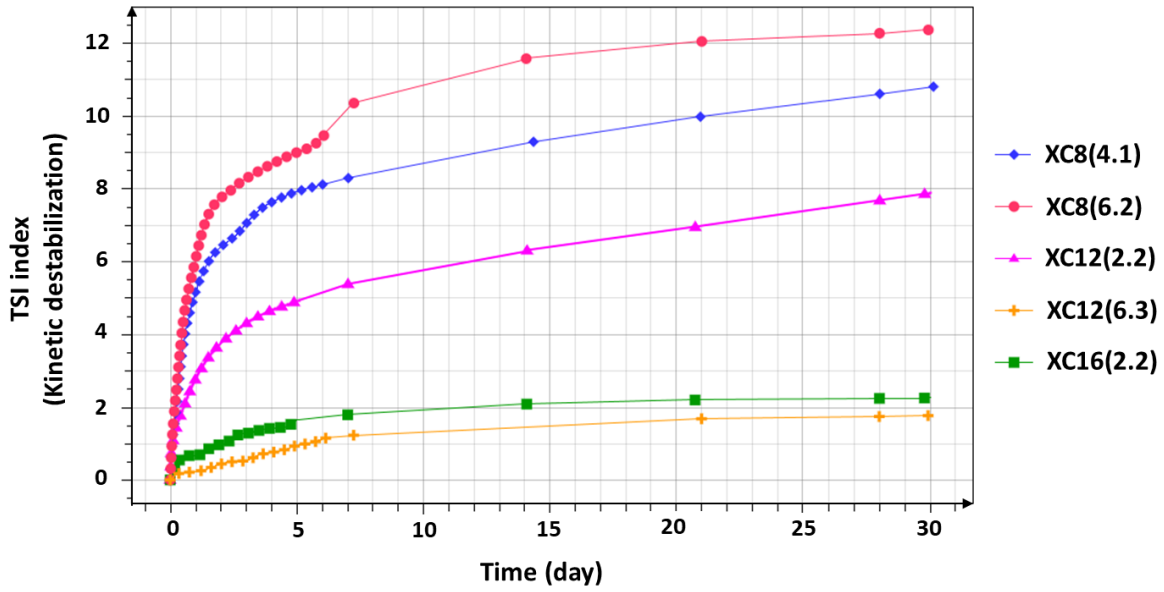

Figure 8: Evolution of the destabilization kinetic represented by the TSI index for emulsion at 0.2% w/w of different HMX

Based on all results of this second section, both parameters: DS and alkenyl chain length appear essential as they sharply govern the emulsions kinetic stability. In fact, the goal for preparing stable emulsion can be achieved using two modified xanthan categories, the dodecenyl grafted xanthan as far as it owns a minimum DS value (over 6%) on the one hand, the hexadecenyl grafted xanthan even at low DS due to long hydrophobic moieties on the second hand. These results are directly related to the surface-active properties, as previously reported for such HMX owning sufficient DS and/or longer alkenyl chains, both demonstrated as the most effective in reducing the surface tension [31]. In addition, such a higher stability may also be facilitated by a negatively more charged polysaccharide due to the presence of anionic carboxylate groups in the linkage between alkenyl chains and the xanthan backbone, this providing repulsive forces between the oil droplets, preventing their aggregation [27,40,41].

It is important to note that all attempts to get stable emulsions with using octenyl grafted xanthan failed even when using HMX owning high DS (up to 6.2%). Thus, in order to improve the emulsion stability, attempts to investigate the effect of HMX concentration on the one hand and the impact of the presence of native xanthan as thickening agent on the other hand were investigated and exposed in the last section of the paper.

3.3. Impact of HMX concentration and effect of synergy between native and modified xanthan

In this third section, XC8(4.7) was used to investigate on the one hand the effect of concentration by increasing from 0.2 to 0.5% w/w and on the other the effect of adding 0.1% w/w of native xanthan

(NX) as thickener in emulsion containing 0.2% w/w of XC8(4.7) as emulsifying agent. In this section emulsions were monitored for several weeks.

Figure 9 presents the macroscopic aspect for the mixtures corresponding to XC8(4.7) at 0.2% w/w (Figure 9.a), XC8(4.7) at 0.5% w/w (Figure 9.b) and mixture prepared with combining XC8(4.7) at 0.2% w/w + Native Xanthan at 0.1% w/w (Figure 9.c). The first one (a) shows no more than ⅓ of the height for the emulsified phase as it was demonstrated in the previous sections. Then, mixtures prepared with increasing HMX concentration to 0.5% are characterized by an entire emulsification remaining stable after several weeks. Same behavior was observed for XC8(4.7) at 0.2% w/w + Native Xanthan (c) system from a macroscopic point of view. Thus, both parameters undoubtedly have marked positive impact on the stability of the dispersions.

In addition, photographs corresponding to D21 superimposed on the granulometric distribution as represented in Figure 9 bring additional information. Afterwards, micrographs show smaller droplets at D21 for emulsions prepared with lonely HMX additive; the higher the HMX content the smaller the droplets of the emulsified phase. Same tendency but in a lesser extent is observed with addition native xanthan. This is confirmed by a droplets size distribution owning smaller droplets and with lower median diameter for corresponding emulsions at both D0 and D21 as visible in Table 3.

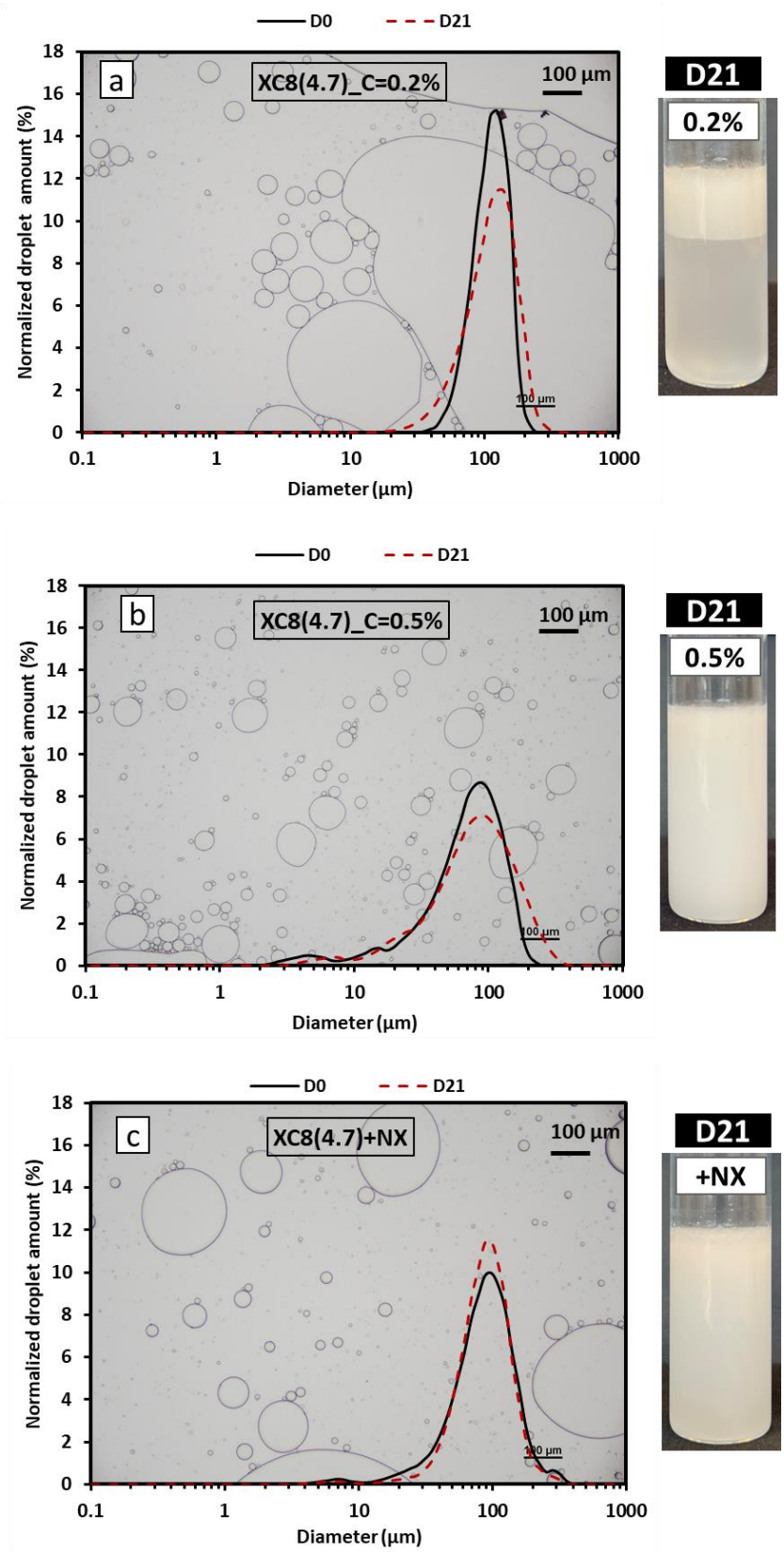

Figure 9 : Photographs at D21, optical microscopy image at D21 and droplet size distribution at D0 and D30 of the emulsified phase of dispersion at different formulation conditions containing XC8(4.7)

Table 3 also gives the evolution over time of mean diameter for each sample. Again, it is obvious that droplets obtained directly after the preparation protocol (D0), are smaller when either adding NX or increasing the HMX concentration, thus illustrating more efficient emulsification. This is consistent

with results obtained elsewhere on OSA-soy polysaccharide [42], inulin-OSA/DDSA [39], OSA-Arabic gum [29,43], and OSA-starch [25,43]. Authors evidenced a droplet size decreasing when increasing the derivative polymer concentration. This was explained as the consequence of higher polymer concentration that makes more emulsifying species available to quickly adsorb on the oil droplets and cover their surface during the homogenization process, thus preventing flocculation.

Then, it was possible to demonstrate that adding NX gives the best stability of mean and median diameter showing constant values 21 days after the emulsion preparation, this is consistent with qualitative results described by Fantou et al. [44] on the positive effect of mixing xanthan grafted by octylamine hydrophobic chains with its pristine xanthan in emulsions stabilization.

Table 3: Median and mean diameter of droplets in the emulsified phase of dispersion at different formulation conditions containing XC8(4.7) at D0 and D21

		XC8(4.7)-C=0.2%	XC8(4.7)-C=0.5%	XC8(4.7)-NX
Median diameter (µm)	**D0**	106 ± 5	70 ± 6	83 ± 4
	D21	109 ± 15	76 ± 3	83 ± 3
Mean diameter (µm)	**D0**	103 ± 6	59 ± 5	78 ± 6
	D21	94 ± 2	68 ± 2	79 ± 2

Thus, as a synergy between the emulsifying agent (HMX) and the thickener one (NX) may play a valuable role for stabilizing dispersion. Backscattering experiments and TSI data were investigated and considered to bring a better understanding of the parameters responsible for such effects. Corresponding results are reported in Figure 10.

For XC8(4.7) at 0.2% w/w content backscattering profile is identical to the one presented in the first section (Figure 4), with highlighting the main occurrence of both creaming and flocculation phenomenon. Then, one can notice that when increasing the HMX concentration from 0.2 to 0.5%, the BS remains globally constant for the whole height of the sample over the entire period of time (Figure 10a). Nevertheless, the absence of perfect superposition for the BS curves may be explained by the presence of large droplets due to local flocculation/coalescence mechanisms without significant creaming migration. Such hypothesis is consistent with the slight increase of both mean and median diameter observed over time (Table 3). From a general point of view, BS profile allows establishing stabilization effect when increasing HMX content, in good accordance with both macroscopic and microstructure aspects.

Finally, if considering the third mixture prepared with HMX/NX combination, one can notice that there is hardly no agreement depending on whether the macroscopic or microscopic aspect is considered; thereby, BS evolution clearly evidences a creaming destabilization profile (Figure 10b). However, one can notice that the destabilization is actually sharply slowed down when compared to the referent emulsion without NX. In fact, a progressive decrease of BS signal for the lower phase is observed at the same time as the increase of BS signal for the upper phase. Indeed, the migration of droplets from the bottom to the top is significantly slowed down when compared to the one in the absence of NX. Thus, despite a macroscopically fully emulsified appearance, BS data allowed revealing a lonely phenomenon of slow creaming rather than a long-term stabilization., Finally, TSI index was considered as a tool to better evidence the differences in the kinetic of destabilization for the three mixtures studied in this paragraph; corresponding graphs are as presented in Figure 10c. This parameter was followed up immediately after the mixture was prepared and monitored for a period of 21 days.

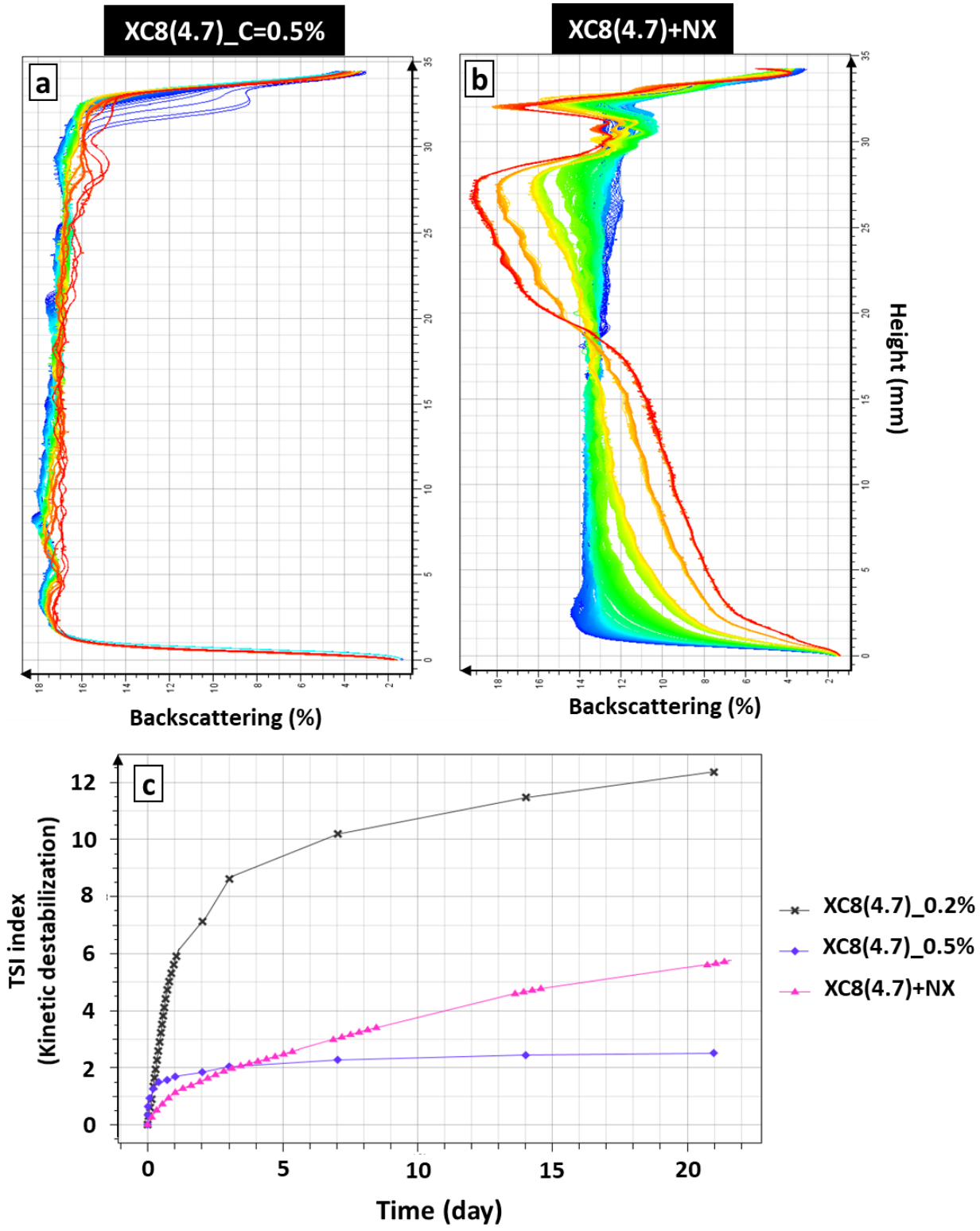

Figure 10: Raw backscattering data (a, b) and TSI index (c) obtained by following the evolution over 21 days of three type of emulsion containing XC8(4.7); the samples were scanned with different scan periods

One can first notice that TSI evolution corresponding to the basic emulsion (C = 0.2%) shows a sharp TSI increase within the first 24 hours after the mixture preparation, after which the increase becomes less rapid but does not tend to stabilize after 21 days. It is interesting to note that TSI index for this mixture remains higher than the others on the entire period of time considered. At the same time, the

addition of NX clearly slows down the destabilization of the mixture with a lower TSI over the whole duration of the study thus highlighting the main action of native xanthan which acts as a thickener delaying the destabilization. Finally, the evolution of TSI corresponding to the emulsion owing the higher HMX content presents a marked tendency to reach an almost constant value (# 2.5) reflecting a stable emulsion; this latter result undoubtedly allows demonstrating the efficacity for amphiphilic xanthan derivatives for the stabilization of O/W emulsions provided there is a sufficient quantity.

Same effects were observed with considering dodecenyl grafted xanthan owning a low grafting rate (4.8%) (results not shown in the paper). In summary, it can be concluded that increasing HMX concentration or adding NX can be considered as efficient solutions to improve O/W emulsion stability over time.

4. Conclusion

In the current work, emulsifying properties of a series of hydrophobically modified xanthan (HMX) have been studied in order to better understand the phenomenon governing the O/W emulsions stability. HMX was obtained via grafting onto xanthan different alkenyl chain length, namely octenyl, dodecenyl and hexadecenyl with grafting densities (DS) up to 16%. First, this work allowed establishing that as amphiphilic polysaccharide derivatives, HMX do own emulsifying properties to stabilize water-oil interfaces while native xanthan do not due to their poor interfacial properties as non-absorbing polymers. High stable emulsions were obtained with HMX grafted either by long alkenyl moieties (XC16) even at low grafting density or by medium chain length (XC12) as far as a minimum DS is used (>6%). These latter HMX allowed getting the best emulsion stability with white and homogeneous aspect, constant size distribution, smaller droplets and constant backscattering profile over one month. Such a promising result can be explained by (1) the effectiveness of HMXs in reducing surface tension and (2) to their increased negative charges due to carboxylate groups providing repulsive forces between droplets thus preventing their aggregation. On the contrary, emulsions prepared with using octenyl grafted xanthan showed destabilization through creaming phenomenon; in this case, it was possible to improve emulsion stability by increasing HMX content, thus providing more amphiphilic chains available to coat oil droplets and prevent creaming. Additional experiments allowed demonstrating that another option to improve the stability of O/W emulsions was mixing HMX and native xanthan together, the latter markedly acting as a thickener for the continuous aqueous phase thus slowing down the creaming of oil droplets. To summarize, the whole of this work undoubtedly brings original knowledge on the role and mechanisms of action of hydrophobically modified xanthan in the stabilization of Oil-in-Water emulsions. The promising and effective emulsifying capacities of biosourced amphiphilic xanthan obtained under green modification synthesis was demonstrated, what offers promising potential for industrial applications. Further investigations are now in progress with the particular aims (1) to elucidate the partitioning of HMX molecules between the bulk aqueous phase and O/W interface and (2) to better understanding the reason for the creaming phenomenon.

Acknowledgments

Authors acknowledge the Region Normandie (Agreement 19E00776) and FEDER for grant and financial support.

Reference

[1] I. Klojdová, J. Štětina, Š. Horáčková, W/O/W Multiple Emulsions as the Functional Component of Dairy Products, Chemical Engineering & Technology. 42 (2019) 715–727. https://doi.org/10.1002/ceat.201800586.

[2] A. Azeem, M. Rizwan, F.J. Ahmad, Z.I. Khan, R.K. Khar, M. Aqil, S. Talegaonkar, Emerging Role of Microemulsions in Cosmetics, Recent Patents on Drug Delivery & Formulation. 2 (2008) 275–289.

[3] Barkat Ali Khan, Basics of pharmaceutical emulsions: A review, Afr. J. Pharm. Pharmacol. 5 (2011). https://doi.org/10.5897/AJPP11.698.

[4] L.A. Trujillo-Cayado, M.C. García, J. Santos, J.A. Carmona, M.C. Alfaro, Progress in the Formulation of Concentrated Ecological Emulsions for Agrochemical Application Based on Environmentally Friendly Ingredients, ACS Sustainable Chem. Eng. 5 (2017) 4127–4132. https://doi.org/10.1021/acssuschemeng.7b00106.

[5] T.F. Tadros, Emulsion Science and Technology: A General Introduction, in: Emulsion Science and Technology, John Wiley & Sons, Ltd, 2009: pp. 1–56. https://doi.org/10.1002/9783527626564.ch1.

[6] M.M. Robins, Emulsions — creaming phenomena, Current Opinion in Colloid & Interface Science. 5 (2000) 265–272. https://doi.org/10.1016/S1359-0294(00)00065-0.

[7] J. Sjöblom, EMULSIONS AND EMULSION STABILITY, Taylor & Francis, 2006. http://www.scientificspectator.com/documents/surfactant%20spectator/Emulsions%20and%20Emulsion%20Stability.pdf.

[8] A. Paraskevopoulou, D. Boskou, V. Kiosseoglou, Stabilization of olive oil – lemon juice emulsion with polysaccharides, Food Chemistry. 90 (2005) 627–634. https://doi.org/10.1016/j.foodchem.2004.04.023.

[9] J.-L. Salager, SURFACTANTS Types and Uses, FiRP, UNIVERSIDAD DE LOS ANDES-Mérida-Venezuela, 2002. http://www.nanoparticles.org/pdf/Salager-E300A.pdf.

[10] Y. Chevalier, M.-A. Bolzinger, Emulsions stabilized with solid nanoparticles: Pickering emulsions, Colloids and Surfaces A: Physicochemical and Engineering Aspects. 439 (2013) 23–34. https://doi.org/10.1016/j.colsurfa.2013.02.054.

[11] E. Dickinson, Hydrocolloids at interfaces and the influence on the properties of dispersed systems, Food Hydrocolloids. 17 (2003) 25–39. https://doi.org/10.1016/S0268-005X(01)00120-5.

[12] Y. Lu, W. Kang, J. Jiang, J. Chen, D. Xu, P. Zhang, L. Zhang, H. Feng, H. Wu, Study on the stabilization mechanism of crude oil emulsion with an amphiphilic polymer using the β-cyclodextrin inclusion method, RSC Adv. 7 (2017) 8156–8166. https://doi.org/10.1039/C6RA28528G.

[13] E. Rotureau, M. Leonard, E. Marie, E. Dellacherie, T.A. Camesano, A. Durand, From polymeric surfactants to colloidal systems (1): Amphiphilic dextrans for emulsion preparation, Colloids and Surfaces A: Physicochemical and Engineering Aspects. 288 (2006) 131–137. https://doi.org/10.1016/j.colsurfa.2006.01.044.

[14] A.K. Ray, P.B. Bird, G.A. Iacobucci, B.C. Clark, Functionality of gum arabic. Fractionation, characterization and evaluation of gum fractions in citrus oil emulsions and model beverages, Food Hydrocolloids. 9 (1995) 123–131. https://doi.org/10.1016/S0268-005X(09)80274-9.

[15] D.R. Underwood, P.S.J. Cheetham, The isolation of active emulsifier components by the fractionation of gum talha, Journal of the Science of Food and Agriculture. 66 (1994) 217–224. https://doi.org/10.1002/jsfa.2740660217.
[16] E.J. Vernon-Carter, R. Pedroza-Islas, C. i. Beristain, Stability of Capsicum Annuum Oleoresin-in-Water Emulsions Containing Prosopis and Acacia Gums, Journal of Texture Studies. 29 (1998) 553–567. https://doi.org/10.1111/j.1745-4603.1998.tb00183.x.
[17] N. Garti, D. Reichman, Surface properties and emulsification activity of galactomannans, Food Hydrocolloids. 8 (1994) 155–173. https://doi.org/10.1016/S0268-005X(09)80041-6.
[18] Y. Wu, W. Cui, N.A.M. Eskin, H.D. Goff, An investigation of four commercial galactomannans on their emulsion and rheological properties, Food Research International. 42 (2009) 1141–1146. https://doi.org/10.1016/j.foodres.2009.05.015.
[19] N. Garti, Z. Madar, A. Aserin, B. Sternheim, Fenugreek Galactomannans as Food Emulsifiers, LWT - Food Science and Technology. 30 (1997) 305–311. https://doi.org/10.1006/fstl.1996.0179.
[20] A.R. Sav, T.A. Meer, R.A. Fule, P.D. Amin, Investigational Studies on Highly Purified Fenugreek Gum as Emulsifying Agent, Journal of Dispersion Science and Technology. 34 (2013) 657–662. https://doi.org/10.1080/01932691.2012.683983.
[21] L.M. Landoll, Nonionic polymer surfactants, Journal of Polymer Science: Polymer Chemistry Edition. 20 (1982) 443–455. https://doi.org/10.1002/pol.1982.170200218.
[22] M. Hennock, R.R. Rahalkar, P. Richmond, Effect of Xanthan Gum Upon the Rheology and Stability of Oil-Water Emulsions, Journal of Food Science. 49 (1984) 1271–1274. https://doi.org/10.1111/j.1365-2621.1984.tb14968.x.
[23] E. Liwarska-Bizukojc, K. Miksch, A. Malachowska-Jutsz, J. Kalka, Acute toxicity and genotoxicity of five selected anionic and nonionic surfactants, Chemosphere. 58 (2005) 1249–1253. https://doi.org/10.1016/j.chemosphere.2004.10.031.
[24] E. Olkowska, Ż. Polkowska, J. Namieśnik, Analytics of Surfactants in the Environment: Problems and Challenges, Chem. Rev. 111 (2011) 5667–5700. https://doi.org/10.1021/cr100107g.
[25] L.A. Bello-Pérez, C.A. Bello-Flores, M. del C. Nuñez-Santiago, C.P. Coronel-Aguilera, J. Alvarez-Ramirez, Effect of the degree of substitution of octenyl succinic anhydride-banana starch on emulsion stability, Carbohydrate Polymers. 132 (2015) 17–24. https://doi.org/10.1016/j.carbpol.2015.06.042.
[26] H.-M. Chen, X. Fu, Z.-G. Luo, Esterification of sugar beet pectin using octenyl succinic anhydride and its effect as an emulsion stabilizer, Food Hydrocolloids. 49 (2015) 53–60. https://doi.org/10.1016/j.foodhyd.2015.03.008.
[27] S. Kokubun, I. Ratcliffe, P.A. Williams, The interfacial, emulsification and encapsulation properties of hydrophobically modified inulin, Carbohydrate Polymers. 194 (2018) 18–23. https://doi.org/10.1016/j.carbpol.2018.04.018.
[28] G. Li, F. Zhu, Physicochemical, rheological, and emulsification properties of nonenyl succinic anhydride (NSA) modified quinoa starch, International Journal of Biological Macromolecules. (2021). https://doi.org/10.1016/j.ijbiomac.2021.10.199.
[29] H. Wang, P.A. Williams, C. Senan, Synthesis, characterization and emulsification properties of dodecenyl succinic anhydride derivatives of gum Arabic, Food Hydrocolloids. 37 (2014) 143–148. https://doi.org/10.1016/j.foodhyd.2013.10.033.

562 [30] S. Plate, S. Diekmann, U. Steinhäuser, S. Drusch, Determination of the degree of
563 substitution of hydrolysed octenylsuccinate-derivatised starch, LWT - Food Science and
564 Technology. 46 (2012) 580–582. https://doi.org/10.1016/j.lwt.2011.12.014.
565 [31] M. Abou Dib, N. Hucher, E. Gore, M. Grisel, Original tools for xanthan hydrophobization
566 in green media: Synthesis and characterization of surface activity, Carbohydrate
567 Polymers. 291 (2022) 119548. https://doi.org/10.1016/j.carbpol.2022.119548.
568 [32] C. Fantou, S. Comesse, F. Renou, M. Grisel, Hydrophobically modified xanthan:
569 Thickening and surface active agent for highly stable oil in water emulsions,
570 Carbohydrate Polymers. 205 (2019) 362–370.
571 https://doi.org/10.1016/j.carbpol.2018.10.052.
572 [33] C. Chauvierre, D. Labarre, P. Couvreur, C. Vauthier, A new approach for the
573 characterization of insoluble amphiphilic copolymers based on their emulsifying
574 properties, Colloid Polym Sci. 282 (2004) 1097–1104. https://doi.org/10.1007/s00396-
575 003-1040-9.
576 [34] Formulaction, Turbiscan Tower User Guide 2.0, (2020).
577 https://formulaction.com/product/turbiscan-tower/.
578 [35] O. Mengual, G. Meunier, I. Cayré, K. Puech, P. Snabre, TURBISCAN MA 2000: multiple
579 light scattering measurement for concentrated emulsion and suspension instability
580 analysis, Talanta. 50 (1999) 445–456. https://doi.org/10.1016/S0039-9140(99)00129-0.
581 [36] M. Luo, X. Qi, T. Ren, Y. Huang, A.A. Keller, H. Wang, B. Wu, H. Jin, F. Li, Heteroaggregation
582 of CeO2 and TiO2 engineered nanoparticles in the aqueous phase: Application of
583 turbiscan stability index and fluorescence excitation-emission matrix (EEM) spectra,
584 Colloids and Surfaces A: Physicochemical and Engineering Aspects. 533 (2017) 9–19.
585 https://doi.org/10.1016/j.colsurfa.2017.08.014.
586 [37] Y. Cao, E. Dickinson, D.J. Wedlock, Creaming and flocculation in emulsions containing
587 polysaccharide, Food Hydrocolloids. 4 (1990) 185–195. https://doi.org/10.1016/S0268-
588 005X(09)80151-3.
589 [38] P.A. Gunning, D.J. Hibberd, A.M. Howe, M.M. Robins, Gravitational destabilization of
590 emulsions flocculated by non-adsorbed xanthan, Food Hydrocolloids. 2 (1988) 119–129.
591 https://doi.org/10.1016/S0268-005X(88)80010-9.
592 [39] S. Kokubun, I. Ratcliffe, P.A. Williams, The emulsification properties of octenyl- and
593 dodecenyl- succinylated inulins, Food Hydrocolloids. 50 (2015) 145–149.
594 https://doi.org/10.1016/j.foodhyd.2015.04.021.
595 [40] M. Falkeborg, Z. Guo, Dodecenyl succinylated alginate (DSA) as a novel dual-function
596 emulsifier for improved fish oil-in-water emulsions, Food Hydrocolloids. 46 (2015) 10–
597 18. https://doi.org/10.1016/j.foodhyd.2014.12.011.
598 [41] D.J. Mcclements, Critical Review of Techniques and Methodologies for Characterization
599 of Emulsion Stability, Critical Reviews in Food Science and Nutrition. 47 (2007) 611–649.
600 https://doi.org/10.1080/10408390701289292.
601 [42] J.-R. Qi, D.-Y. Zhang, R.-X. Zhao, J.-S. Liao, Y. Cao, J. Xiao, Micro- and nano- emulsions
602 based on soluble soy polysaccharide and octenyl succinic anhydride modified soluble soy
603 polysaccharide, International Journal of Food Science & Technology. n/a (2020).
604 https://doi.org/10.1111/ijfs.14947.
605 [43] Z. Wang, D. Ma, L. Gan, X. Lu, Y. Wang, Octenyl succinate esterified gum arabic stabilized
606 emulsions: Preparation, stability and in vitro gastrointestinal digestion, LWT. 149 (2021)
607 112022. https://doi.org/10.1016/j.lwt.2021.112022.

[44] C. Fantou, J. Yoo, S. Comesse, M. Grisel, F. Renou, Synergy between pristine and hydrophobically modified xanthans for stabilizing oil-in-water emulsions, Colloid and Interface Science Communications. 37 (2020) 100292. https://doi.org/10.1016/j.colcom.2020.100292.